JCI The Journal of Clinical Investigation

The Concentration and Significance of the Butanol-Extractable I^{131} of Serum in Patients with Diverse States of Thyroidal Function

Sidney H. Ingbar, … , Paul D. Hoeprich, John W. Athens

J Clin Invest. 1954;33(3):388-399. https://doi.org/10.1172/JCI102911.

Research Article

THE CONCENTRATION AND SIGNIFICANCE OF THE BUTANOL-EXTRACTABLE I^{131} OF SERUM IN PATIENTS WITH DIVERSE STATES OF THYROIDAL FUNCTION

By SIDNEY H. INGBAR,[1] NORBERT FREINKEL,[1] PAUL D. HOEPRICH, AND JOHN W. ATHENS

(From the Department of Biophysics, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C.)

(Submitted for publication September 28, 1953; accepted November 12, 1953)

Thyroxine, if not truly the active thyroid hormone (1, 2) is clearly the principal iodine-containing compound released by the thyroid gland, and ordinarily comprises the largest fraction of the plasma's protein-bound iodine (3–6). Many investigators have, therefore, attempted to assess thyroidal function by measuring the concentration of protein-bound I^{131} (PBI^{131}) in the plasma following the administration of radioactive iodine (7–11). The PBI^{131} (12), or even the total radioactivity in the plasma (13, 14), determined several days after the administration of the tracer, reflects the radioactivity of circulating thyroxine, except in patients who have received large doses of I^{131} (15).

It has been shown that the hormonal iodine which is extractable from serum with butanol and not re-extractable with alkali (hereafter referred to as the butanol-extractable iodine) is composed largely of thyroxine. Its concentration in serum differs significantly from that of the protein-bound iodine (16).

The present communication reports the results in 109 patients with varying states of thyroidal function in whom the plasma's concentration of radioactive butanol-extractable iodine was determined both one and three days after the administration of I^{131}. From a correlation of these results with measurements of the amount of I^{131} accumulated by the thyroid gland, an estimate of both the amount and the rate of turnover of glandular hormone has been made.

MATERIALS AND METHODS

The present study includes all patients referred to the Department of Biophysics, Army Medical Service Graduate School, Walter Reed Army Medical Center during the period between January, 1952 and June, 1952 for the determination of 24-hour thyroidal uptake of radioactive iodine. Since no attempt was made to select cases within this group, which included dependents as well as military personnel, the population studied is probably representative of that referred to the thyroid clinic of any large hospital. Each patient was questioned and examined by one or another of the authors, and a diagnostic impression was recorded prior to the administration of I^{131}. This record, together with the result of the iodine-uptake test, was reviewed by one of the authors (S. H. I.), and each patient was assigned a definite diagnosis before the results of the butanol-extraction procedure were known. Prolonged observation of the patients, in an attempt to ascertain the accuracy of the diagnosis, was usually impossible.

A standard amount (50 to 100 μc) of I^{131}, free of carrier, was administered orally to each patient. The patient returned 24 hours later for the determination of the thyroidal uptake of the tracer. This was carried out with a shielded Geiger-Mueller tube (bismuth-sputtered-cathode) placed 20 cm. from the neck. The radioactivity in the neck was compared with that of a 50 ml. volumetric flask, similarly positioned, containing an amount of I^{131} equal to the dose administered. In most patients the thyroidal content of I^{131} was also determined 48 and 72 hours after the administration of the tracer. The following convention was adopted as an index of the preponderance of either accumulation of radioiodine or the release of radioactive hormone during the interval between 24 and 72 hours after the administration of I^{131}. When the thyroidal uptake displayed a progressive increase during this period, the 24-hour uptake was subtracted from the 72-hour uptake and the difference ("net change") was given a positive sign. When the uptake decreased at any time during the period, the lowest value was subtracted from the highest and the difference was given a negative sign.

During the 24 and 72-hour visits, blood was drawn for the measurement of the concentration of butanol-extractable I^{131} (BEI^{131}). Separation of the serum was carried out as soon as clot retraction had occurred. In virtually all cases, the analysis was begun on the same day the blood was drawn.

The method used in the extraction of thyroxine from serum was an adaptation of that described by Man, Kydd, and Peters (16). Five cc. of serum were brought to a pH of approximately 3.5 with 10 per cent H_2SO_4, using

[1] Present address: Thorndike Memorial Laboratory, Boston City Hospital, Boston 18, Mass.

a few drops of bromphenol-blue as an internal indicator. Twenty-five cc. of n-butyl alcohol were then added in a fine stream, while the serum was continuously agitated. The resulting mixture was centrifuged at 2500 r.p.m for 8 minutes. The supernatant butanol was transferred to a 125 ml. centrifuge separatory funnel. The precipitated protein was successively re-extracted with 20 cc. and 15 cc. portions of butanol. The three butanol extracts were combined within the separatory funnel, vigorously extracted three times with 60 cc. of Blau's solution (4 N NaOH containing 5 per cent Na_2CO_3). Slow centrifugation (300 r.p.m.) for 5 minutes was used to break the emulsion which invariably formed. The alkalinized, washed butanol extract was transferred to a 150 cc. distilling flask and was distilled to dryness *in vacuo* at a temperature not exceeding 40°C. The dried residue was dissolved in 1.5 to 2.0 cc. of butanol or water and transferred to weighed planchets. Dilute HCl was added in order to reduce the alkalinity, and thereby to diminish the hygroscopic properties of the mixture. When the residue was taken up in water, an immiscible layer appeared, which contained almost all the bromphenol-blue and most of the thyroxine. Radioactivity in this phase was 3 to 8 times as great as in the aqueous phase. The chemical nature of this immiscible layer has not been determined. It does not originate from serum, since it can be demonstrated when saline, with or without added thyroxine, is extracted, and presumably, therefore, arises from either the apparatus or the reagents.

In the determination of the concentration of BEI^{131} in erythrocytes, blood was drawn into a tube containing dried heparin. The erythrocytes were separated from the plasma and washed three times with normal saline solution. Five cc. of packed cells were laked with distilled water. Concomitant analyses for the concentration of BEI^{131} in plasma and laked erythrocytes were then carried out as above.

The radioactivity of the samples was measured with an end-windowed (mica 1.4 to 1.8 mg. per cm.^{2}) Geiger-Mueller tube and an automatic sample changer. At least 24,000 total counts were obtained for each serum sample. A correction, based on mass, was made in the observed radioactivity of each sample. Since, in the average euthyroid patient, the total thyroxine radioactivity in each sample of serum was approximately two-thirds of background radiation, approximately 9500 disintegrations were observed. The probable error of the determined radioactivity was therefore approximately 1 per cent. The accuracy of the counting procedure was considerably greater in hyperthyroid patients, in whom the concentration of radioactive thyroxine was usually markedly increased.

RESULTS

In an attempt to evaluate the analytic procedure described above, the recovery from serum of radioactive potassium iodide, diiodotyrosin,[2] and thyroxine[2] was determined (Table I). From 88 to 97 per cent of radioiodine added as thyroxine was recovered from the final residue, whereas I^{131} added as either potassium iodide or diiodotyrosine was virtually excluded.

[2] Obtained from Abbott Laboratories, Chicago, Illinois.

TABLE I

Recovery of radioactive thyroxine, diiodotyrosine, and potassium iodide during butanol-extraction procedure

Radioactive substance added	Number of experiments	Per cent of added radioactivity in butanol extract*		
		Prior to alkaline wash	Following alkaline wash	Following distillation
		%	%	%
Thyroxine	8	99.4	97.2	94.3
Diiodotyrosine	4	47.1	0.05	0.02
Potassium iodide	6	80.5	0.03	0.01

* Average values.

In both butanol-acetic acid-water and collidine-ammonia systems (17), filter paper chromatography revealed that the radioactive material isolated from the serum of three patients who had received therapeutic doses of I^{131} moved precisely as did synthetic radiothyroxine. Both the material extracted from serum and synthetic radiothyroxine displayed small amounts of radioactivity with R.F.'s corresponding to those of iodide and diiodotyrosine. Degradation of thyroxine during the chromatographic procedure, leading to the appearance of similar fractions, has been previously described (17).

The recovery from serum of triiodothyronine was not determined. Furthermore the chromatographic systems employed would probably not have permitted resolution of this material from thyroxine. The data, therefore, offer no evidence concerning the extent, if any, to which radioactive triiodothyronine contributed to the observed values of the BEI^{131}.

The concentration of BEI^{131} was determined in the washed erythrocytes of three patients who had received therapeutic doses of radioactive iodine.

TABLE II

Lack of transport of radioactive thyroxine in human erythrocytes

Patient	BEI^{131}	BEI^{131}
	(% dose/liter plasma)	(% dose/liter RBC)
1	.301	.000
2	.203	.000
3	.214	.002

TABLE III—*Summary of values obtained in diverse states of thyroidal function*

Patient	Age	Sex	24-Hour BEI^{131} (% *dose/liter*)	72-Hour BEI^{131} (% *dose/liter*)	24-Hour thyroidal I^{131} uptake (% *dose*)	24–72 Hour change in I^{131} uptake* (% *dose*)	BMR (% *normal*)
			Euthyroidism				
1	59	F	.009	.062	41	− 0.8	− 5
2	37	F	.012	.036	33		
3	35	M	.016	.035	37	− 0.4	−16
4	42	F	.014	.038	29	+ 1.1	+ 4
5	29	F	.008	.023	25	+ 8.6	− 8
6	43	F	.011	.033	19	+ 4.9	−21
7	35	F	.020	.063	28	− 1.9	
8	38	F	.006	.020	30	+ 4.6	−20
9	27	F	.014	.043	40	+ 2.3; −2.5	−10
10	26	F	.020	.047	39	+ 2.1	− 2
11	29	F	.022	.046	26	+ 2.0; −1.6	−17
12	58	F	.010	.030	34	− 1.3	−29
13	25	F	.010	.028	29	− 0.2	+ 3
14	37	F	.012	.044	35	+ 2.2	−20
15	29	M	.008	.022	17	+ 0.7	+ 8
16	59	M	.018	.040	36	+ 2.2; −1.9	+22
17	51	F	.018	.032	23	+ 0.5	+ 4
18	41	F	.032	.078	40	+ 1.0; −1.3	+ 3
19	37	F	.017	.051	32	+ 2.2	−26
20	27	F	.045	.079	30	+ 3.6; −3.3	+ 2
21	47	F	.019	.039	20	− 0.6	+ 6
22	41	F	.013	.032	32	+ 0.4; −0.2	
23	28	F	.023	.028	20	+ 2.3; −1.5	− 5
24	55	F	.018	.040	20	+ 1.9	
25	40	F	.028	.050	21	+ 0.1	− 5
26	38	F	.020	.057	43	− 5.4	−28
27	44	M	.010	.038	24	+ 1.9; −2.2	−33
28	52	M	.012	.017	29	+ 1.4	−10
29	44	F	.005	.041	28	+ 0.8; −1.0	
30	33	F	.010	.033	31	+ 4.0; −2.0	+ 6
31	32	F	.014	.054	30	− 1.0	−11
32	43	F	.030	.053	32	− 0.6	−18
33	58	M	.004	.015	37	+ 7.8	−30
34	27	F	.014	.043	37	+ 3.2; −1.3	+ 7
35	57	F	.005	.016	32	+ 3.4; −1.5	
36	32	F	.016	.015	32	+ 2.0	− 5
37	35	F	.010	.053	44	+ 0.5; −0.7	+15
38	31	F	.022	.036	39	+ 2.0; −0.7	+19
39	49	F	.008	.020	14	+ 0.5; −0.2	− 4
40	50	F	.041	.067	49	+ 0.6; −0.3	−30
41	33	F	.025	.067	32	− 0.5	−13
42	55	F	.012	.051	39	+ 0.7	
43	27	F	.013	.020	57	+ 1.8	− 4
44	30	F	.008	.030	29	+ 0.9	−14
45	71	F	.018	.021	18	+ 1.9	− 6
46	48	F	.014	.021	34	+ 2.5; −1.5	
47	30	M	.008	.019	18	+ 0.2; −1.2	−24
48	33	M	.022	.042	25	+ 2.0	−23
49	48	M	.010	.062	26	+ 6.0; −4.0	−21
50	36	F	.007	.018	13	− 1.2	− 6
51	15	F	.033	.044	24	+ 2.3	− 9
52	38	F	.017	.035	34	− 5.4	−29
53	31	F	.014	.020	55		+ 2
54	62	F	.010	.024	27	− 0.2	− 8
55	33	F	.012	.038	26	+ 0.6	+ 4
56	38	F	.010	.022	17	+ 3.4	+ 4
57	28	F	.012	.014	40	− 0.8	
58	32	F	.016	.035	23		0
59	28	F	.018	.018	16		
60	28	M	.020	.039	18		+ 5
61	32	F	.014	.041	44	+ 5.3; −2.0	+13
62	35	M	.009	.024	26		
63	30	M	.015	.052	25		−10
64	28	M	.012	.032	21		−12
		Mean	.016	.037	30	+ 0.1	
		Std. Dev.	.008	.016	9	2.6	

* Two figures given in patients demonstrating Pattern No. 2 (see Text). The negative value in these cases is used in calculations.

TABLE III—*Continued*

Patient	Age	Sex	24-Hour BEI^{131} (% dose/liter)	72-Hour BEI^{131} (% dose/liter)	24-Hour thyroidal I^{131} uptake (% dose)	24–72 Hour change in I^{131} uptake* (% dose)	BMR (% normal)
Hyperthyroidism							
65	37	F	.747	1.085	80	−18.5	+50
66	22	M	.018	.023	65	− 1.2	+37
67	46	F	.151	.338	94	−22.1	+41
68	29	M	.036	.204	76	− 6.7	+35
69	22	F	.568	.712	96	− 5.6	+50
70	28	F	.019	.250	38	− 3.2	+37
71	44	F	.053	.127	77	− 4.9	+42
72	42	F	.085	.150	97	− 4.4	+55
73	60	M	.141	.308	66	+ 9.3; −4.1	
74	53	F	.198	.468	65	+ 2.0; −2.7	+28
75	58	F	.202	.549	75	−25.8	+37
76	29	M	.034	.253	57	− 4.7	+49
77	28	M	.104	.283	95	− 9.7	+40
78	51	F	.230	.548	69	−16.3	+72
79	30	F	.104	.429	48	+10.4; −2.0	+30
80	41	F	.081	.327	56	+ 0.2; −2.0	+33
81	37	F	.054	.163	55	+ 6.0; −2.5	+30
82	35	F	.850	1.170	76	− 6.0	+62
83	27	F	.488	1.040	94		
84	37	F	.340	.862	76	−12.3	+55
85	28	F	.019	.079	42	− 0.4	+18
86	23	M	.047	.080	56	+ 3.3	+30
87	41	F	.166	.301	80	− 3.2	+42
88	59	F	.020	.081	45		+25
89	62	F	.359	.611	82		+38
		Mean	.205	.418	70	− 7.0	
		Std. Dev.	.232	.104	18	7.2	
Thyroidal Inactivity							
90†	32	F	.002	.002	2		−38
91‡	23	M	.003	.004	19		−28
92‡	24	M	.004	.006	17		−30
93†	28	F	.004	.003	10		−35
94†	14	F	.006	.008	2		−30
95§	35	F	.002	.006	1		
96§	36	M	.010	.011	7		+15
97†	45	F	.003	.002	1		−38
98†	50	F	.004	.004	2		−40
99†	28	F	.006	.007	2		−36
100‡	47	M	.006	.006	4		−25
101‡	55	F	.003	.008	4		−27
102†	30	F	.006	.005	3		−45
103§	36	M	.004	.008	9		
		Mean	.004	.006	6		
		Std. Dev.	.002	.003	6		
Post-Thyroidectomy ‖							
104	24	F	.318	.066	7		−38
105	8	F	.224	.159	4		−30
105a¶	8	F	.162	.040	4		
106	29	M	.121	.078	3		−35
106a	29	M	.090	.083	4		
107	18	F	.088	.025	2		−37
108	28	M	.010	.037	2		−29
108a	28	M	.060	.048	9		
109	40	F	.072	.080	15		−25
109a	40	F	.069	.086	21		
		Mean	.121	.070	7		
		Std. Dev.	.091	.038	6		

† Primary myxedema.
‡ Hypopituitarism.
§ Euthyroid receiving exogenous thyroid.
‖ All patients in this group underwent thyroidectomy for thyroidal carcinoma.
¶ Repeat test in same patient.

Although simultaneously determined concentrations of BEI^{131} in the plasma were high, the radioactivity extracted from the erythrocytes was indistinguishable from background radiation (Table II).

In all euthyroid and hyperthyroid patients, the concentration of BEI^{131} in the serum increased during the interval between 24 and 72 hours after the administration of the tracer (Table III). Patients with decreased thyroidal function associated with primary or pituitary myxedema, or with the ingestion of thyroid substance shall hereafter be referred to as patients with thyroidal inactivity. In this group of cases, significant differences between the 24 and 72 hour concentrations could not be detected. In five of six patients with carcinoma of the thyroid in whom total thyroidectomy had been attempted, and in two patients with thyrotoxicosis who continued to take propylthiouracil throughout the period of observation (not shown in Table III) the 24-hour concentration of BEI^{131} exceeded that found at 72 hours.

The mean values of the BEI^{131} concentrations in patients with thyrotoxicosis, euthyroidism, or thyroidal inactivity differed significantly from that found in the other two groups ($p < .01$), both at the 24-hour and at the 72-hour period. However, a better separation between the values found in patients with thyroidal inactivity, euthyroidism, and hyperthyroidism was found at the 72-hour period (Figure 1). In only one patient presumed to have thyrotoxicosis did the 72-hour concentration of BEI^{131} fall definitely within the normal range. This patient (No. 66) was a 22-year old male with severe exophthalmos, goiter, a thyroidal radioiodine uptake of 65 per cent at 24 hours, and very mild symptoms of thyrotoxicosis. In three patients in whom the diagnosis of thyrotoxicosis had been made, the BEI^{131} values were just above the range of normal. Of these patients one (No. 86) was a young male with exophthalmos, goiter, a 56 per cent radioiodine uptake, and minimal symptoms of thyrotoxicosis. The other two patients (No. 85, No. 88) were anxious women in whom the diagnosis of thyrotoxicosis was in doubt at the outset.

In one patient (No. 96) with thyroidal inactivity, the BEI^{131} value fell at the lower end of the normal range. This patient was a 36-year old male with non-toxic nodular goiter, who was receiving 2 gr. of dessicated thyroid substance daily, and whose 24-hour radioiodine uptake was 7 per cent.

In hyperthyroid patients, the mean net change in thyroidal uptake between 24 and 72-hours (− 7.0 per cent) was significantly different ($p < .01$) from that found in euthyroid subjects (+ 0.1 per cent). This was also true when the net change was calculated in each patient as percentage of the 24-hour radioactive iodine uptake. (− 9.0 per cent *vs.* + 0.6 per cent). Successive determinations of thyroidal uptake at 24, 48, and 72 hours disclosed three patterns. The first was a progressive increase in the thyroidal content of radioiodine, the second an increase followed by a decrease, and the third a successive decrease. Of the euthyroid patients, 41 per cent demonstrated pattern 1, 36 per cent, pattern 2, and 23 per cent pattern 3. Of the hyperthyroid patients, 4.6 per cent (one patient) demonstrated pattern 1, 18.2 per cent pattern 2, and 77.2 per cent pattern 3. The severity of thyrotoxicosis could not be consistently correlated with the pattern of consecutive neck counts. However, those patients with the most severe thyrotoxicosis generally demonstrated pattern 3, while those with the mildest disease displayed either pattern 1 or a small net loss of radioactivity of the type found in pattern 2.

In patients with decreased thyroidal function, the low level of radioactivity in the neck precluded the accurate measurement of progressive changes

TABLE IV

Correlation between the functions studied in various clinical groups

Functions	r	p
24 Hr. BEI^{131} *vs.* 72 Hr. BEI^{131}		
Euthyroid	0.59	<.001
Hyperthyroid	0.91	<.001
Combined*	0.63	<.001
72 Hr. BEI^{131} *vs.* 24 Hr. thyroidal uptake		
Euthyroid	0.23	>.05
Hyperthyroid	0.35	>.05
Combined	0.66	<.001
72 Hr. BEI^{131} *vs.* "Net change"†		
Euthyroid	−0.34	<.01
Hyperthyroid	−0.44	<.02
Combined	−0.40	<.001
24 Hr. thyroidal uptake *vs.* "Net change"		
Euthyroid	−0.06	>.05
Hyperthyroid	−0.20	>.05
Combined	−0.61	<.001

* Euthyroid and hyperthyroid groups considered together.

† 24–72 Hour change in thyroidal I^{131} uptake.

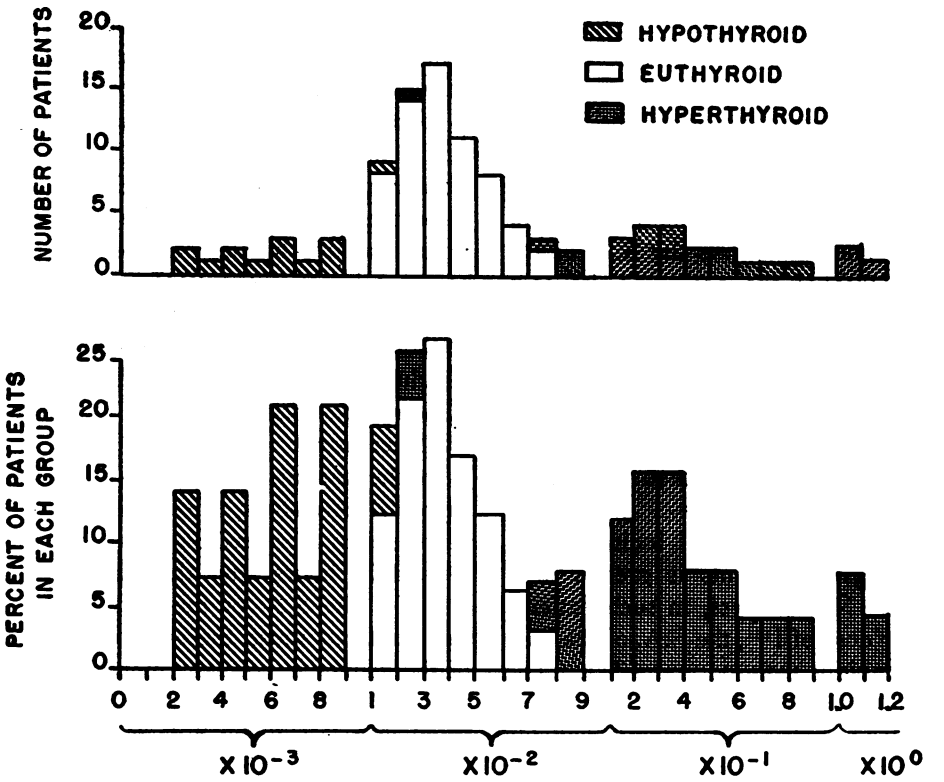

FIG. 1. DISTRIBUTION OF 72-HOUR CONCENTRATIONS OF BUTANOL-EXTRACTABLE I^{131} IN THE SERUM OF HYPERTHYROID, EUTHYROID, AND HYPOTHYROID PATIENTS (EXCLUDING PATIENTS WITH THYROIDAL CARCINOMA)

in the uptake of radioactive iodine. Nevertheless, it appeared certain that some thyroidal accumulation and storage of I^{131} had occurred, since measureable radioactivity persisted in the region of the thyroid gland for several days.

Statistical correlations between the various functions studied are presented in Table IV.

DISCUSSION

Recovery experiments have demonstrated that the extraction procedure described is relatively specific for the principal iodine-containing compound in the plasma, thyroxine. In confirmation of previous reports, thyroxine has been found to be completely absent from the erythrocyte (18).

Measurement of the concentration of radioactive thyroxine in the serum following the administration of a standard dose of radioiodine has been found to be a rather reliable guide to the state of thyroidal function in patients with thyrotoxicosis, euthyroidism, and thyroidal inactivity. In several patients who had undergone virtually complete thyroidectomy, however, the concentration of BEI^{131} did not correlate well with the clinical status of the patient. An explanation for this discrepancy was sought in an analysis of the factors determining the concentration of BEI^{131} in the plasma.

The principal and perhaps only source of plasma's thyroxine appears to be the pool of hormonal iodine within the thyroid gland. Therefore, the relationship described by Zilversmit, Entenman, and Fishler for a product substance and its immediate precursor can be applied to the relationship existing between thyroxine in the plasma and in the thyroid gland (19). These authors have demonstrated that when the precursor, and thence the product, is labelled with a radioactive component, the specific radioactivity (radioactive substance

X/total substance X) of the precursor initially exceeds that of the product. The specific activity (S.A.) of the product then rises with a rapidity which is, at any time, proportional to the magnitude of the difference between the S.A.'s of the precursor and the product. The peak S. A. of the product is reached when the specific activities of the two have become equal. As the S.A. of the product exceeds that of the precursor, its own S.A. begins to decline. If it be assumed that the content of non-radioactive hormonal iodine in the thyroid gland and in the plasma remain constant during the period of observation, then changes in the radioactivity of the thyroid gland or of the plasma's BEI will be proportional to changes in their respective S.A.'s.

The concentration of hormonal iodine in the plasma of hyperthyroid patients is greater than normal (20, 21). Nevertheless, in such patients following a tracer dose of I^{131}, the S.A. of the PBI increases more rapidly than normal (22). The present findings suggest that the rate of increase of the S.A. of the BEI is also augmented in patients with thyrotoxicosis. In such patients, the S.A. of the glandular hormonal iodine must therefore exceed, at least initially, that found in patients with normal thyroidal function. The relatively greater S.A. of the thyrotoxic gland commonly results from an increase in the initial accumulation of I^{131} and from a reduction in the total quantity of hormone stored within the gland (23).

The "net change" in glandular radioactivity describes the direction and magnitude of changes in the S.A. of organically-bound iodine within the gland during the 24 to 72-hour interval. A decrease in the S.A. of the glandular hormone [8] was seen more often in thyrotoxic than in normal patients. The average thyroidal S.A. increased by 0.6 per cent in normal patients, but it decreased by 9 per cent in thyrotoxic patients. The more rapid loss of radioactivity from the thyroid glands of thyrotoxic patients results from an increase in the rapidity of disappearance of radioiodide from the plasma (24) which hastens the cessation of collection of radioiodine, and from an increase in the rate of turnover of glandular hormone, as will be seen below.

Thus in thyrotoxic patients, the S.A. of the plasma's hormone is increasing and that of the glandular hormone is decreasing more rapidly than normal. The time interval required for the two to become equal and for the concentration of BEI^{131} to reach a peak, should therefore be shorter than normal in thyrotoxic patients. This proves to be the case when re-utilization of radioiodide made available by the peripheral degradation of hormone is prevented (22). However, when as in the present study, re-utilization of radioiodide is allowed to occur, the decline in the S.A. of the glandular hormone is retarded, the peak concentration of the plasma's BEI^{131} is delayed, and its level increased.

In the patients "totally" thyroidectomized for thyroidal carcinoma, the concentration of BEI^{131} in the serum decreased during the interval between 24 and 72 hours after the administration of the tracer. The S.A. of the BEI in the plasma of these patients must, therefore, have exceeded that of the glandular hormone more quickly than normal. This could be explained by either a more rapid decrease in the S.A. of the glandular hormone, a more rapid increase in the S.A. of the plasma's hormone, or both. All patients who demonstrated this pattern were hypothyroid, and in three the PBI was found to be subnormal. Since these patients demonstrated a normal or increased concentration of BEI^{131} in their serum at 24 hours, it may be concluded that the S.A. of their circulating hormonal iodine had increased more rapidly than normal. However, the presence in the plasma of normal or increased concentrations of BEI^{131} suggests that rather large amounts of radioactive hormone had been released. Furthermore, the hypothyroid state of these patients indicates that the total amount of hormone released by their thyroid tissue per unit time was subnormal. Thus the S.A. of the hormone released by these glands must have been greater than normal. This conclusion, together with the markedly decreased initial uptake of I^{131} found in the thyroid glands of these patients, indicates that the radioactive hormone formed was minimally "diluted" by preformed, non-radioactive hormone. The total amount of hormone stored by these thyroid glands must, therefore, have been considerably decreased.

A similar decrease in the concentration of BEI^{131} between 24 and 72 hours after the administration of the dose was noted in the serum of two pa-

[8] The term "hormone," used in this sense, refers to all iodinated tyrosyl compounds within the gland.

tients with thyrotoxicosis made euthyroid by the administration of propylthiouracil. This agent reduces the rate of formation of thyroid hormone (25, 26) but does not diminish, and may rather increase the rate of release of hormone from the gland (27). In the hyperthyroid patient, this must result in a marked depletion of glandular hormone.

It is apparent that the concentration of BEI^{131} in the serum does not always accurately reflect the overall rate of hormone formation or release. For any given rate of radioiodine accumulation, depletion of glandular hormone results in an increased release of BEI^{131}. To the extent that depletion of glandular hormone occurs in patients with thyrotoxicosis, the sensitivity of the test in detecting the hyperthyroid state is increased. However, when depletion of glandular hormone occurs in patients who are not thyrotoxic, the concentration of BEI^{131} in the serum does not correlate well with the clinical state. Two cases of postoperative myxedema demonstrating this phenomenon have recently been reported (28).

It is possible to evaluate further the manner in which the amount of glandular hormone influences the concentration of BEI^{131} in the serum. It will be assumed that the maximal accumulation of radioiodine within the thyroid gland is instantaneous, and that within the first three days after the administration of the tracer, peripheral utilization of released radioactive hormone is negligible.[4]

If A = thyroidal content of I^{131} at time, t (per cent of administered dose).

A_0 = the peak thyroidal accumulation of I^{131} (per cent of administered dose).

(BEI^{131}) = concentration of BEI^{131} in the serum at time, t (per cent dose/liter serum).

V = volume of distribution of BEI^{131} (total extrathyroidal $BEI^{131}/(BEI^{131})$.

r = rate of turnover of glandular hormone (per cent of glandular hormone synthesized or released per day).

t = time after peak thyroidal accumulation of I^{131} (days).

The amount of I^{131} within the thyroid gland at any time,

$$A = A_0e^{-rt}$$

The rate of release of radioactive hormone,

$$\frac{d(BEI^{131})V}{dt} = rA_0e^{-rt}$$

Therefore, the total amount of BEI^{131} released up to time, t,

$$(BEI^{131})V = -A_0e^{-rt} + C$$

Since when $t = 0$, $(BEI^{131})\ V = 0$,

$$C = A_0$$

and

$$(BEI^{131})V = A_0(1 - e^{-rt}) \qquad (1)$$

At any time, and for any value of V and A_0, the concentration of BEI^{131} in the serum will depend upon the rate of turnover of glandular hormone (r). Therefore, at any given rate of hormone manufacture, decreases in the quantity of glandular hormone increase the concentration of BEI^{131} in the serum by increasing the rate of turnover of hormone within the thyroid gland.

Unlike the thyroid tissue of patients designated as having thyroidal inactivity, that of the patients who had been thyroidectomized is characterized by a high rate of turnover of glandular hormone. It is not known whether, in these patients the remaining thyroid tissue is normal or neoplastic in character. If it were the latter, then the increased turnover rate could be the result of an inability of the tissue to store iodine because of a structural characteristic, such as absence of follicles (29). It is more likely, however, that the increased turnover rates reflect an intense stimulation of the residual thyroid tissue by thyrotropin (30). In the latter instance, the failure of the residual tissue to maintain a euthyroid state could result solely from an inadequate glandular mass. In either case, attempts to alter the function of these thyroidal remnants in such a manner as to make possible therapy with radioactive iodine might well be directed towards increasing the thyroidal storage

[4] It is, of course, recognized that the maximal thyroidal accumulation of I^{131} is not instantaneous. Furthermore, it is likely that considerable utilization of radioactive hormone occurs during the 72-hour period of observation, especially in patients with thyrotoxicosis. Nevertheless, the mathematical relationships defined are considered to be first approximations to those which truly pertain to the functions under consideration.

of hormone as well as increasing the avidity for iodine.

Equation 1 suggests that it may be possible to evaluate the rate of turnover of glandular hormone by examining the relationship between the BEI^{131} and the initial accumulation of radioiodine within the thyroid gland. From equation 1, the slope of the curve representing the relationship between these two functions may be described as

$$\frac{d(BEI^{131})}{dA_0} = \frac{(1 - e^{-rt})}{V} \qquad (2)$$

For any value of t, the slope will be a straight line, provided that both V and r are constant.

Figure 2 illustrates the relationship observed in the present study between the concentration of BEI^{131} in the serum 72 hours after the administration of the tracer and the 24-hour thyroidal uptake. The values displayed by patients with thyroidal carcinoma, although shown on the chart, are not included in the calculations made from this figure. In each of the other patients, the observed 24-hour thyroidal uptake of radioiodine is "corrected" for the amount of radioactive hormone which has been released by this time. This correction is made by adding to the observed uptake the product of the 24-hour concentration of BEI^{131} in the serum and an assumed volume of distribution of BEI^{131} of 24 liters (27). The curve shown is the visual best fit to a series of points representing the average value of all patients within each 10 per cent uptake-interval. Rather than following a straight line, the slope of the curve rises sharply as the uptake approaches 100 per cent. Since the volume of distribution of thyroid hormone apparently does not differ among patients with varying states of thyroidal function (27), equation 2 indicates that as the maximal uptake increases, the rate of turnover of glandular hormone increases. This conclusion is in accord with direct measurements, which reveal an increased rate of release of hormone from thyrotoxic glands (31).

The curve shown in Figure 2 is described by the equation

$$\text{Uptake} = \frac{100(BEI^{131})}{.09 + (BEI^{131})} \qquad (3)$$

However, the relationship between the thyroidal rate of clearance of plasma iodide and the uptake of radioactive iodine has been described previously by the following equation (32):

$$\text{Uptake} = \frac{100 \times \text{thyroidal iodide clearance } (C_t)}{\text{thyroidal clearance } (C_t) + \text{renal clearance } (C_r)} \qquad (4)$$

Therefore, from equations 3 and 4,

$$\text{Uptake} = \frac{100(BEI^{131})}{.09 + (BEI^{131})} = \frac{100C_t}{C_t + C_r} \qquad (5)$$

$$(BEI^{131}) = \frac{.09\,C_t}{C_r} \qquad (6)$$

If the average normal rate of renal clearance of iodide is assumed to be 2.0 liters per hour (33),

$$(BEI^{131}) = .045\,C_t \qquad (7)$$

where C_t = thyroidal iodide clearance rate (liters per hour).

The BEI^{131} concentration in the serum of patients with hyperthyroidism, euthyroidism, and thyroidal inactivity, therefore, appears to be directly proportional to the thyroidal rate of clearance of plasma radioiodide. This conclusion is in accord with the *a priori* assumption that the thyroidal rate of accumulation of iodide is equal to the rate of release of hormonal iodine. Employing equation 7, and the average normal value for the BEI^{131} found in the present study, a thyroidal clearance rate of 0.78 liters per hour may be derived, which is in accord with direct measurements of this function (33–35). From equation 1, together with equations 3, 4, and 7, it is possible to estimate the average rate of turnover of thyroidal hormone for any value of the thyroidal clearance rate, the 72-hour BEI^{131}, or thyroidal uptake of radioactive iodine. It can be shown (see Appendix) that

$$-r = \frac{\ln (.9784 - .0108\,C_t)}{3} \qquad (8)$$

$$-r = \frac{\ln (.9784 - .24(BEI^{131})}{3} \qquad (9)$$

$$-r = \frac{\ln \left(1 - \frac{2.16}{100 - A_0}\right)}{3} \qquad (10)$$

From equation 9, the turnover rate associated with the average normal value of the BEI^{131} found in the present study is approximately 1 per cent per day. There is considerable evidence that the aver-

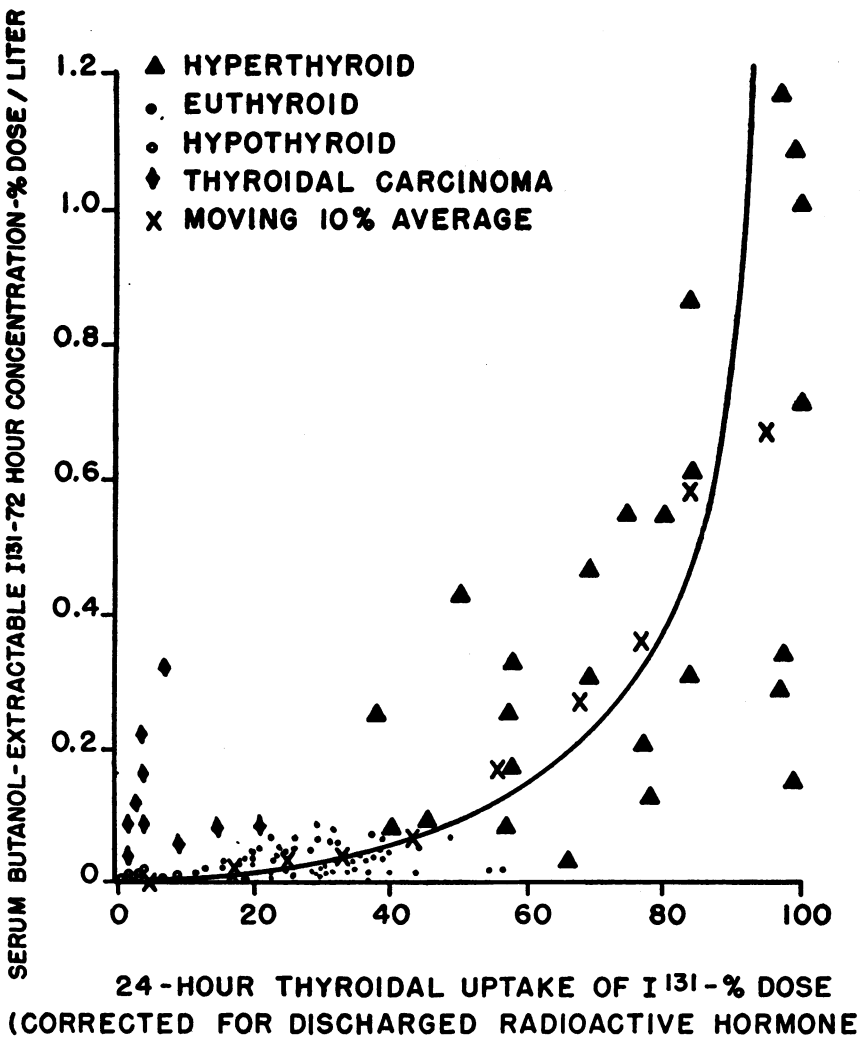

FIG. 2. THE RELATIONSHIP BETWEEN 24-HOUR THYROIDAL UPTAKES OF I^{131} AND 72-HOUR CONCENTRATIONS OF BUTANOL-EXTRACTABLE I^{131} IN SERUM

Thyroidal uptakes have been "corrected" by adding to the observed uptake the quantity of radioactive hormone released during this interval (24-hour BEI^{131} × assumed volume of distribution of 24 liters). The curve shown represents the visual best fit to a series of moving averages of 10 per cent-uptake intervals. Values obtained in patients with thyroidal carcinoma have been excluded from calculations of interval-averages.

age normal daily production of thyroxine is approximately 70 mcg. daily (27). The hormone content of the average normal thyroid gland can, therefore, be estimated to be approximately 7000 mcg., which value agrees closely with those obtained by direct chemical analysis (36, 37).

SUMMARY

1. A method for the determination of the BEI^{131} of serum has been shown to measure principally the radioactivity of circulating thyroxine, and has been applied to the study of the concentration of BEI^{131} in the serum of 109 patients with various states of thyroidal function.

2. These results have been correlated with the 24-hour thyroidal uptake of radioiodine and with the subsequent change in thyroidal radioiodine content during the 24–72 hour period.

3. Analysis of the results reveals that the concentration of BEI^{131} in the serum is dependent on

the initial thyroidal uptake of I^{131}, the rate of synthesis of thyroid hormone, and the amount of hormone stored within the gland.

4. The 72-hour concentration of BEI^{131} correlates well with the clinical state except in nonthyrotoxic patients in whom the quantity of hormone stored within the gland is markedly decreased.

ACKNOWLEDGMENTS

The authors are greatly indebted to Mrs. Mary McLaughlin and Maj. Margaret E. Peters, ANC, for invaluable technical assistance.

APPENDIX

From equation 1 (see Text),

$$(BEI^{131})V = A_0(1 - e^{-rt})$$

substituting for (BEI^{131}) and A_0 from equations 4 and 7

$$.045\, C_tV = \frac{100\, C_t}{C_t + C_r}(1 - e^{-rt})$$

where V = 24 liters, C_r = 2 liters per hour, and t = 3 days,

$$1.08\, C_t = \frac{100\, C_t}{C_t + 2}(1 - e^{-3r})$$

$$1.08\, C_t^2 + 2.16\, C_t = 100\, C_t(1 - e^{-3r})$$

$$.0108\, C_t + .0216 = 1 - e^{-3r}$$

$$e^{-3r} = .9784 - .0108\, C_t$$

$$-r = \frac{\ln (.9784 - .0108\, C_t)}{3}$$

Furthermore, from equation 7,

$$C_t = \frac{(BEI^{131})}{.045}$$

Therefore,

$$-r = \frac{\ln .9784 - .24\,(BEI^{131})}{3}$$

According to equation 3,

$$A_0 = \frac{100(BEI^{131})}{.09 + (BEI^{131})}$$

$$.09\, A_0 + A_0(BEI^{131}) = 100(BEI^{131})$$

$$(BEI^{131}) = \frac{.09\, A_0}{100 - A_0}$$

Therefore, according to equation 1,

$$\frac{.09\, A_0V}{100 - A_0} = A_0(1 - e^{-3r})$$

$$\frac{.09 \times 24}{100 - A_0} = 1 - e^{-3r}$$

$$\frac{2.16}{100 - A_0} = 1 - e^{-3r}$$

$$e^{-3r} = 1 - \frac{2.16}{100 - A_0}$$

$$-r = \frac{\ln\left(1 - \frac{2.16}{100 - A_0}\right)}{3}$$

REFERENCES

1. Gross, J., and Pitt-Rivers, R., The identification of 3:5:3'-L-triiodothyronine in human plasma. Lancet, 1952, 1, 439.
2. Gross, J., and Pitt-Rivers, R., Physiological activity of 3:5:3'-L-triiodothyronine. Lancet, 1952, 1, 593.
3. Taurog, A., and Chaikoff, I. L., The nature of the circulating thyroid hormone. J. Biol. Chem., 1948, 176, 639.
4. Laidlaw, J. C., Nature of the circulating thyroid hormone. Nature, 1949, 164, 927.
5. Rosenberg, I. N., The nature of the circulating thyroid hormone in Graves' disease. J. Clin. Invest., 1951, 30, 1.
6. Leblond, C. P., and Gross, J., The mechanism of the secretion of thyroid hormone. J. Clin. Endocrinol., 1949, 9, 149.
7. Freedberg, A. S., Ureles, A., and Hertz, S., Serum level of protein bound radioactive iodine (I^{131}) in the diagnosis of hyperthyroidism. Proc. Soc. Exper. Biol. & Med., 1949, 70, 679.
8. Clark, D. E., Moe, R. H., and Adams, E. E., The rate of conversion of administered inorganic radioactive iodine into protein-bound iodine of plasma as an aid in the evaluation of thyroid function. Surgery, 1949, 26, 331.
9. Seed, L., Jaffé, B., and Baumeister, C., The tracer dose of radioactive iodine in the diagnosis of thyroid disease. J. Clin. Endocrinol., 1951, 11, 1143.
10. Sheline, G. E., and Clark, D. E., Index of thyroid function: estimation by rate of organic binding of I^{131}. J. Lab. & Clin. Med., 1950, 36, 450.
11. Goodwin, J. F., Macgregor, A. G., Miller, H., and Wayne, E. J., The use of radioactive iodine in the assessment of thyroid function. Quart. J. Med., 1951, 20, 353.
12. Harsha, W. N., Evaluation of the conversion of radioactive inorganic iodine to protein-bound iodine as a diagnostic aid in thyroid dysfunction. J. Clin. Endocrinol., 1951, 11, 1524.
13. Silver, S., Fieber, M. H., and Yohalem, S. B., Blood levels after tracer doses of radioactive iodine in the diagnosis of thyroid disorders. Am. J. Med., 1952, 13, 725.

14. Barry, M. C., and Pugh, A. E., Serum concentrations of radioiodine in diagnostic tracer studies. J. Clin. Endocrinol., 1953, 13, 980.
15. Robbins, J., Rall, J. E., Becker, D. V., and Rawson, R. W., The nature of the serum iodine after large doses of I^{131}. J. Clin. Endocrinol., 1952, 12, 856.
16. Man, E. B., Kydd, D. M., and Peters, J. P., Butanol-extractable iodine of serum. J. Clin. Invest., 1951, 30, 531.
17. Taurog, A., Tong, W., and Chaikoff, I. L., The monoiodotyrosine content of the thyroid gland. J. Biol. Chem., 1950, 184, 83.
18. Riggs, D. S., Lavietes, P. H., and Man, E. B., Investigations on the nature of blood iodine. J. Biol. Chem., 1942, 143, 363.
19. Zilversmit, D. B., Entenman, C., and Fishler, M. C., On the calculation of "turnover time" and "turnover rate" from experiments involving the use of labelling agents. J. Gen. Physiol., 1943, 26, 325.
20. Salter, W. T., Bassett, A. M., and Sappington, T. S., Protein-bound iodine in blood: VI. Its relation to thyroid function in 100 clinical cases. Am. J. Med. Sc., 1941, 202, 527.
21. Rapport, R. L., and Curtis, G. M., The clinical significance of the blood iodine: a review. J. Clin. Endocrinol., 1950, 10, 735.
22. Becker, D. V., Rall, J. E., Peacock, W., and Rawson, R. W., The effect of a thyrotrophic hormone preparation on the metabolism of radioiodine in euthyroid, hyperthyroid and acromegalic individuals. J. Clin. Invest., 1953, 32, 149.
23. Gutman, A. B., Benedict, E. M., Baxter, B., and Palmer, W. W., The effect of administration of iodine on the total iodine, inorganic iodine, and thyroxine content of the pathological thyroid gland. J. Biol. Chem., 1932, 97, 303.
24. McConahey, W. M., Keating, F. R., Jr., and Power, M. H., The behavior of radioiodine in the blood. J. Clin. Invest., 1949, 28, 191.
25. Astwood, E. B., Mechanisms of action of various antithyroid compounds. Ann. New York Acad. Sc., 1949, 50, 419.
26. Pitt-Rivers, R., Mode of action of antithyroid compounds. Physiol. Rev., 1950, 30, 194.
27. Riggs, D. S., Quantitative aspects of iodine metabolism in man. Pharmacol. Rev., 1952, 4, 284.
28. Blom, P. S., and Terpstra, J., High PBI^{131} concentration in blood of patients with myxedema: preliminary report. J. Clin. Endocrinol., 1953, 13, 989.
29. Dobyns, B. M., and Maloof, F., The study and treatment of 119 cases of carcinoma of the thyroid with radioactive iodine. J. Clin. Endocrinol., 1951, 11, 1323.
30. Goldsmith, R. E., Stanbury, J. B., and Brownell, G. L., The effect of thyrotropin on the release of hormone from the human thyroid. J. Clin. Endocrinol., 1951, 11, 1079.
31. Freedberg, A. S., Chamovitz, D. L., and Kurland, G. S., Thyroid function in normal and pathological states as revealed by radioactive iodine studies. I. Thyroid I^{131} uptake and turnover in euthyroid, hyperthyroid and hypothyroid subjects. Metabolism, 1952, 1, 26.
32. Myant, N. B., and Pochin, E. E., The thyroid clearance rate of plasma iodine as a measure of thyroid activity. Proc. Roy. Soc. Med., 1949, 42, 959.
33. Myant, N. B., Pochin, E. E., and Goldie, E. A. G., The plasma iodide clearance rate of the human thyroid. Clin. Sc., 1949, 8, 109.
34. Berson, S. A., Yalow, R. S., Sorrentino, J., and Roswit, B., The determination of thyroidal and renal plasma I^{131} clearance rates as a routine diagnostic test of thyroid dysfunction. J. Clin. Invest., 1952, 31, 141.
35. Ingbar, S. H., The simultaneous measurement of the iodide-concentrating and protein-binding capacities of the human thyroid gland. Tr. Am. A. Study Goiter, In press.
36. McClendon, J. F., Iodine and the Incidence of Goiter, Minneapolis, University of Minnesota Press, 1939.
37. Leland, J. P., and Foster, G. L., Cited by Riggs, D. S., Pharmacol. Rev., 1952, 4, 327.